Fungal spore fragmentation as a function of airflow rates and fungal generation methods

Hussein Kanaani[a], Megan Hargreaves[b], Zoran Ristovski[a] and Lidia Morawska[a,*]

[a]International Laboratory for Air Quality and Health, Queensland University of Technology, Brisbane, QLD, Australia

[b]School of Life Sciences, Microbiology Section, Queensland University of Technology, Brisbane, QLD, Australia and International Laboratory for Air Quality and Health

Abstract

The aim of this study was to characterise and quantify the fungal fragment propagules derived and released from several fungal species (*Penicillium*, *Aspergillus niger* and *Cladosporium cladosporioides*) using different generation methods and different air velocities over the colonies. Real time fungal spore fragmentation was investigated using an Ultraviolet Aerodynamic Particle Sizer (UVASP) and a Scanning Mobility Particle Sizer (SMPS). The study showed that there were significant differences ($p < 0.01$) in the fragmentation percentage between different air velocities for the three generation methods, namely the direct, the fan and the fungal spore source strength tester (FSSST) methods. The percentage of fragmentation also proved to be dependant on fungal species. The study found that there was no fragmentation for any of the fungal species at an air velocity ≤ 0.4 m/s for any method of generation. Fluorescent signals, as well as mathematical determination also showed that the fungal fragments were derived from spores. Correlation analysis showed that the number of released fragments measured by the UVAPS under controlled conditions can be predicted on the basis of the number of spores, for *Penicillium* and *Aspergillus niger,* but not for *Cladosporium cladosporioides*. The fluorescence percentage of fragment samples was found to be significantly different to that of non-fragment samples ($p < 0.0001$) and the fragment sample fluorescence was always less than that of the non-fragment samples. Size distribution and concentration of fungal fragment particles were investigated qualitatively and quantitatively, by both UVAPS and SMPS, and it was found that the UVAPS was more sensitive than the SMPS for measuring small sample concentrations, and the results obtained from the UVAPS and SMAS were not identical for the same samples.

Keywords: fungal spores; fragments; air velocity; fluorescent percentage

---

*Corresponding Author. Tel.: + 61-7-3138-2616; Fax: +61-7-3138-9079

E-mail address: l.morawska@qut.edu.au

1. Introduction

Many published studies have confirmed the presence of indoor microbial contamination around the world (Pei-Chih, Huey-Jen et al. 2000; Hargreaves, Parappukkaran et al. 2003; Portnoy, Barnes et al. 2004; Jo and Seo 2005). It has been reported that fungal spores are the cause of many adverse health outcomes, such as asthma, rhinitis, allergies and hypersensitivity pneumonitis (Vijay, Thaker et al. 1999), as well as infectious diseases such as dermatomycoses and aspergillosis (Smith 1976). It has also been found that submicrometer fungal fragments have stronger adverse effects than fungal spores because they can penetrate deeper into the respiratory tract and deposit into bronchi, bronchioles and alveoli (Cho, Seo et al. 2005).

Fungal spore release depends on several factors, including air velocity (air-flow rate over the surface), relative humidity, temperature, building materials, fungal species, ventilation, human activity and the age of mold growth. For example, Kildesø et al. (2003) found that the relationship between released fungal spore numbers and air velocity depends on fungal species, while Pasanen et al. (1991) found that spore release depends on fungal genus and Górny et al. (2001) found that the release of fungal spores is affected by fungal species, air velocity over the surface, surface texture and vibration of the contaminated material.

In terms of fungal fragmentation, many studies have also investigated the potential release and measurement of fungal propagules (Górny, Reponen et al. 2001; Górny, Mainelis et al. 2003; Górny 2004; Cho, Seo et al. 2005). While Cho et al. (2005) investigated the aerodynamic characteristics of the released fungal fragments, using a fungal spore source strength tester, other studies investigated the effects of variables such as fungal species, airspeed, humidity, vibration and colony structures, on the

release of fungal fragments (Górny, Reponen et al. 2001; Górny, Mainelis et al. 2003; Górny 2004).

Passive spore release, as a result of external forces such as air currents and gravity is the most common release mechanism for fungal spores in indoor environments (Gregory 1973; Pasanen, Pasanen et al. 1991; Madelin and Madelin 1995). Since air currents have been identified as a major factor responsible for spore release (Burnett 1976), it is expected that indoor airflow velocities will also play an important role in this release, and subsequently it may play a role in fungal fragmentation.

Airflow in indoor environments depends on the specific conditions of the environment. For example, Handa and Pietrzyk (1996) showed that the airflow velocities for mixed or displacement ventilation ranged from 0 to 0.3 m/s. Thorshauge (1982) found that the mean air speeds in typically ventilated spaces, such as offices and lecture rooms, were in the range 0.05-0.40 m/s, and Matthews et al. (1989) found the median air speed in four typical residence houses was 0.06-0.16 m/s when the central fan was working and 0.02-0.06 m/s when it was off. In order to account for outdoor air velocity (1.4-5.8 m/s (Górny, Reponen et al. 2001)), as well as mechanical and natural ventilation, the air velocities used in this study ranged from 0.1-10.2 m/s.

While previous studies have investigated the effect of air velocity on fungal spore aerosolization (Górny, Mainelis et al. 2003; Górny 2004), they did not cover a wide range of air velocities. The effect of different fungal releasing methods on fungal fragment release is yet to be studied and it also remains unclear exactly which part of the fungus acts as the source for fragment particles during the process of fungal fragmentation. As such, the aim of this study was to characterise and quantify the released fungal fragment propagules from different fungal strains (*Penicillium*,

Aspergillus and Cladosporium), using different releasing methods and air velocities. The spore fragmentation mechanisms were also investigated.

2. Materials and methods

2.1 Fungal preparation

The fungal genera chosen (*Aspergillus*, *Penicillium* and *Cladosporium*) were those frequently occurring indoors, in both Australia and other places in the world (Solomon 1976; Kuo and Li 1994; Burge, Pierson et al. 2000; Pei-Chih, Huey-Jen et al. 2000; Hargreaves, Parappukkaran et al. 2003; Jo and Seo 2005). Each of the *Penicillium* strain (Australian Collection of Microorganisms - ACM 4616) and *Aspergillus niger* (American Type Culture Collection - ATCC 9142) was inoculated onto Sabouraud Dextrose Agar plates (SDA), incubated at 25^{o}C for two weeks and then refrigerated for one day before being used. Because the culture obtained during the two week incubation period for *Cladosporium cladosporioides* (Food Research Laboratory, CSIRO, Australia - FRR 5106) was not sufficient to conduct the experiments properly, it was inoculated on SDA and incubated at 25^{o}C for a further 3 weeks, and these samples were subsequently referred to as having a "high fluorescent percentage" (see below) .

In order to investigate the difference in fluorescent percentage between fragmented and non-fragmented samples, cultures with both "medium and low fluorescent percentages" were also used for *Aspergillus* and *Penicillium*. As the fluorescent percentage of fungal spores is known to decrease as they age, as well as with an increased frequency of air exposure (Kanaani, Hargreaves et al. 2007), these cultures were obtained as follows: after using the high fluorescent percentage cultures for each

species, the agar plates containing fungal cultures were refrigerated for two months and then used again for generating fungal aerosols with a medium fluorescent percentage. After using these medium fluorescent percentage cultures, the agar plates were refrigerated for another two months and then used again for generating fungal aerosols with low fluorescent percentage.

2.2. Instrumentation and the applied protocol

The tests were conducted at the International Laboratory for Air Quality and Health (ILAQH) at Queensland University of Technology, inside a Class II, Type A, Biological Safety Cabinet (SG-400 SterilGARD, Westinghouse Pty Ltd., Australia) over a period of two years. Ten trials (each consisting of nine 3 hour experiments) were conducted for each species of high percentage fluorescent level fungi, with one experiment for each of the three methods of fungal particle generation. These trials used the real time Ultraviolet Aerodynamic Particle Sizer (UVAPS, model 3312, TSI Inc, St. Paul., MN) for detecting and measuring fungal particles (spores and fragments), in order to investigate the effects of using different releasing methods, along with different air velocities, on the characterization, spore fragmentation mechanism and concentration of the released fungal fragments from different fungal species. A further seven trials (consisting of six 2.5 hour experiments, each) were also conducted for *Penicillium* and *Aspergillus* with high percentage fluorescent level only at each of the three methods, using the UVAPS, along with a TSI Model 3071A Scanning Mobility Particle Sizer (SMPS) (TSI Inc., St. Paul, MN, USA) and a TSI Model 3025 Condensation Particle Counter (CPC) (TSI Inc., St. Paul, MN, USA). These trials investigated full scale fragment characterisation at two air velocities (0.4 m/s and 5.5 m/s), which were chosen because 0.4 m/s represents the maximum air

velocity of ventilated spaces (see 'Introduction') and 5.5 m/s was the highest common air velocity for the three methods.

Another seven trials (consisting of twenty one 1 hour experiments, each) were also conducted to investigate the difference in fluorescent percentage between fragment and non-fragment samples. The trials for *Penicillium* and *Aspergillus* were conducted for all percentage fluorescent levels, while for *Cladosporium* they were only conducted for the high percentage fluorescent level. In these experiments, high, medium and low fluorescent percentage cultures of each species were investigated using the UVAPS. All of the trials were conducted using two air velocities (0.4 m/s and 5.5 m/s).

For each experiment, the background was measured using a petri dish containing only SDA. In all cases, the SDA background showed a fluorescence of 0%, with very low concentration levels (0 - 0.01 #/cm^3), and as such, it was not necessary to control for the background. The UVAPS sample time was 20 seconds for all of the experiments where the UVAPS was used alone, and 120 seconds for the remaining trials, which also used an SMPS, also with a sample time of 120 seconds. The UVAPS was calibrated using standard particles, covering a range of 0.93 -7.17 μm aerodynamic diameter, as described in Kanaani et al. (2007). The UV laser pulse energy and photomultiplier tube (PMT) were kept constant during the study (50 ± 1% and 482 V respectively).

While the UVAPS can provide real-time concentrations, size distributions and fluorescence for particles with aerodynamic diameters of 0.5-15 μm, the SMPS measures particle size distribution and number concentration in the range 0.015-0.750 μm. Full scale of airflow velocity measurements were carried out using a constant-temperature, hot-wire anemometry air velocity meter (Model 8330-M-GB

VelociCheck Air Velocity Meter, TSI Inc., Cardigan Road, MN, USA), with an accuracy of ± 5% of reading or ± 0.025 m/s. The temperature and relative humidity range recorded during the study were 23-26^{o}C and 65-69%, respectively.

2.3. Fungal aerosol generation methods

Three dishes of each species were tested per day, one for each of the three methods of generation (as outlined below).

2.3.1. Generation of fungal aerosols by the direct method

The fungi were aerosolised directly from the cultures growing on the agar plates, which were placed inside the mixing chamber (Figure 1a). During the experiments, continuous HEPA-filtered air was directed over the surface of the mycelia at an angle of 60^{o} and a distance of 1.5 cm. Flow rates of 0.5, 2, 5, 9, 14, 20, 25 and 30 L/min (which correspond to 0.1, 0.4, 1.8, 3.3, 5.3, 7.1, 8.5, and 10.2 m/s) were used. Sampling by the UVAPS and/or UVAPS and SMPS (Figure 1) were conducted from the same sampling points within the chamber during all experiments, in order to measure fungal propagule sizes, concentration and fluorescent and total particle counts. The chamber used in the study was 100×39×39 cm and was made of aluminium, except for one side, which was made of Perspex and used as a door.

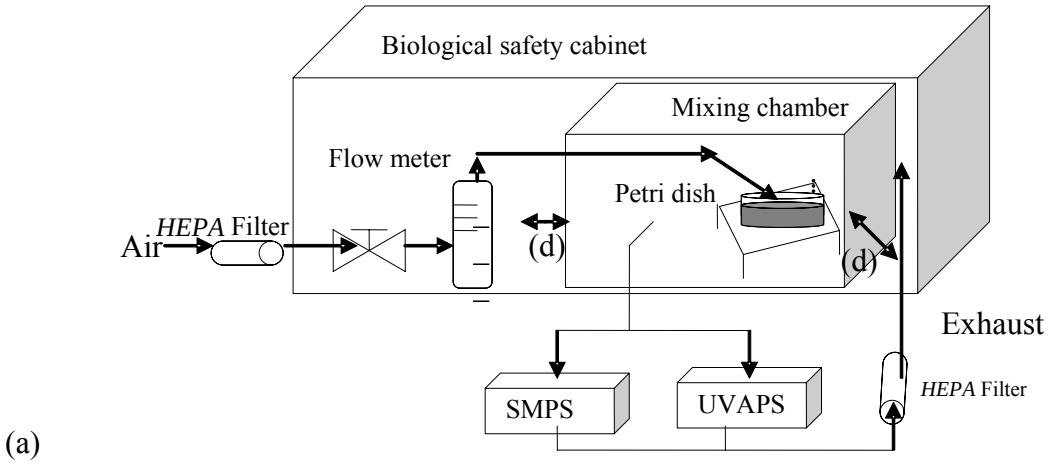
2.3.2. Generation of fungal aerosols using the fan method

A small 12 volt fan, fixed on the top of one side of the chamber (from the inside), was used to generate fungal particles (Figure 1b). The agar plate was held in place by a stand and was directed towards the fan, so that they were both at the same level and that the air hit the fungal colonies directly. The distance between the culture surface and the fan was 2, 5, 15, 30 and 40 cm, in order to get the air blowing on the culture at different speeds (0.1, 0.4, 1.8, 3.3 and 5.3 m/s, respectively). In order to measure air speed, an additional door was constructed for the mixing chamber, which contained

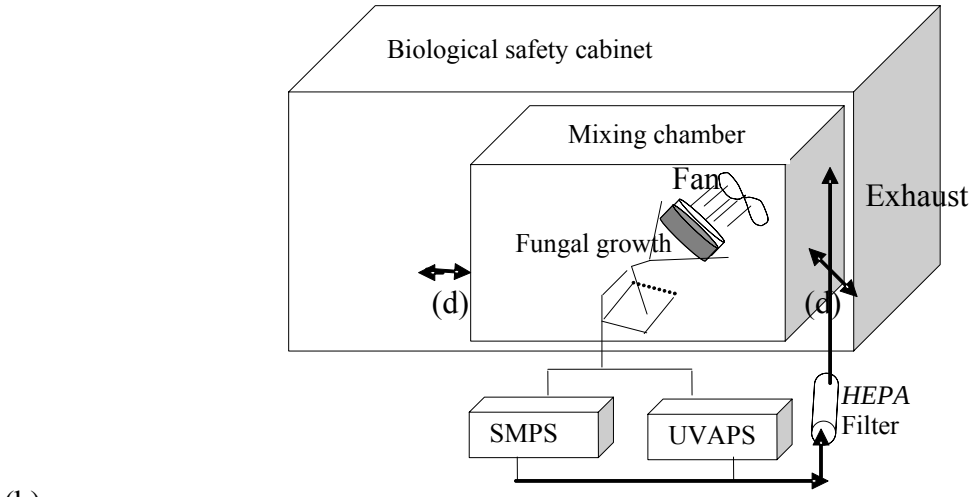
five separately opening holes to allow for the Air Velocity Meter to be inserted at different distances from the Petri dish.

2.3.3. Generation of fungal particles using the FSSST method

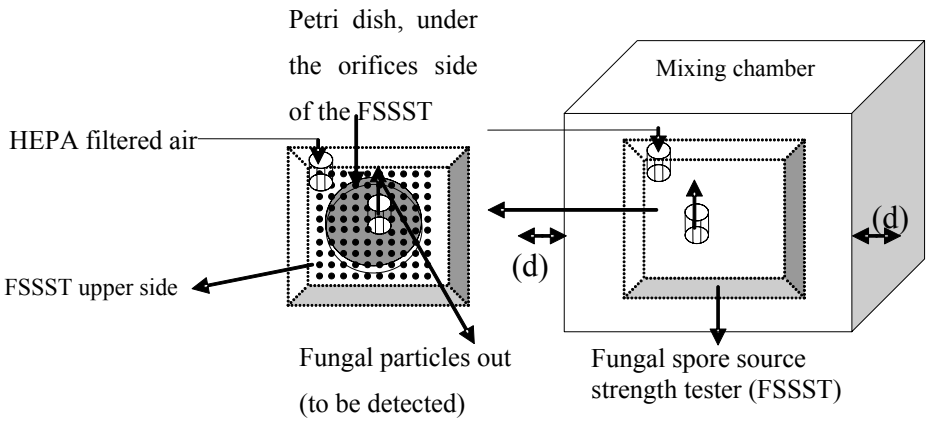
A small device, namely the fungal spore source strength tester (FSSST), was used to release fungal particles (Figure 1c). FSSST is a portable device designed by Sivasubramani et al. (2004) and it is used to generate fungal particles by hitting their colonies with clean air. The FSSST is a small four sided box, with an internal cross-sectional area of 9.5 x 9.5 cm. It is made of polyvinyl chloride, with a 1 cm edge of foam rubber around the open edge. It covers the petri dish and forms a seal with it rim, so that no air can move in or out of the device, other than by the inlet and outlet. The clean, HEPA-filtered air then enters the FSSST via a single opening, and exits via the 112 orifices, so that it is dispersed over the entire area of the fungal dish, which is located directly under the device. The released fungal particles then leave the FSSST via the outlet on its upper surface and are dispersed into the mixing chamber, to be collected and analysed by the UVAPS and/or SMPS. The airflow rates used were 5, 10, 15, 25 and 30 L/min (corresponding to 0.1, 0.4, 1.8, 3.3, and 5.3 m/s, respectively), representing different indoor environments (mechanical ventilation, domestic fan ventilation, natural ventilation). For more information about the FSSST, see Grinshpun et al. (2002) and Sivasubramani et al. (2004).



(a)



(b)



(c)

Figure 1. Experimental set-up for fungal aerosolisation: (a) Direct method; (b) Fan method; (c) Fungal spore source strength tester (FSSST) method (biological safety cabinet, SMPS, UVAPS and HEPA filters are not shown – they are the same as in (a)); (d) Pressure equalizing holes.

2.4. Data analysis

All statistical analysis, such as correlation and regression analysis were conducted using Microsoft Office Excel 2003. Since the sample size (number of trials) was small (10 and 7), which is less than 15, the Mann-Whitney U test was run instead of t-test. Because of the potential effects of non-normality and a modest sample size on the ANOVA F Statistic, the Kruskal-Wallis non-parameter test was also run to compare sample median scores across the groups. The software package- SPSS for Windows version 16.0 was used to conduct these tests. A level of significance p=0.05 was used for all statistical procedures.

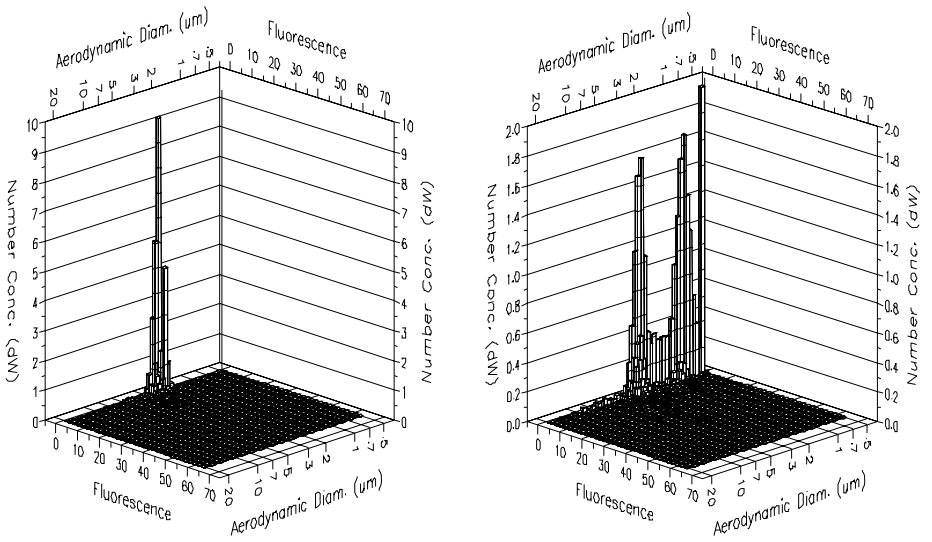
3. Results and Discussion

All figures and tables presented in this study represent the results of high fluorescent percentage culture, except Table 3, which also includes the medium and low fluorescent percentage results.

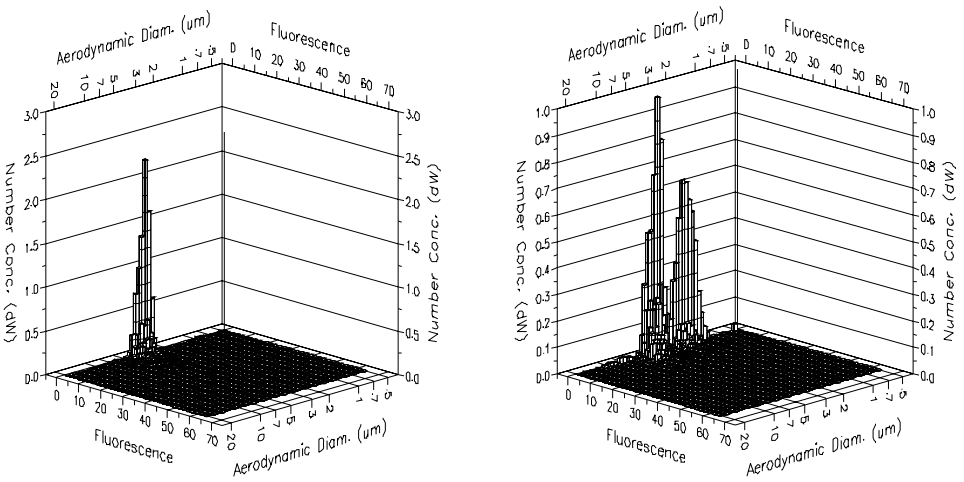
3.1. Released fungal fragment particles as a function of air velocity and generation methods

In all three generation methods (direct, fan and FSSST), fragmentation samples were detected for all three forms of fungi under investigation (*Penicillium*, *Aspergillus* and *Cladosporium*) (Figures 2). As shown in Figure 2, the fragmented fungal samples consisted of particles smaller than those detected in non-fragmented samples for the same species. This finding is consistent with the findings of previous studies that investigated fungal spore fragmentation (Górny, Reponen et al. 2002; Cho, Seo et al.

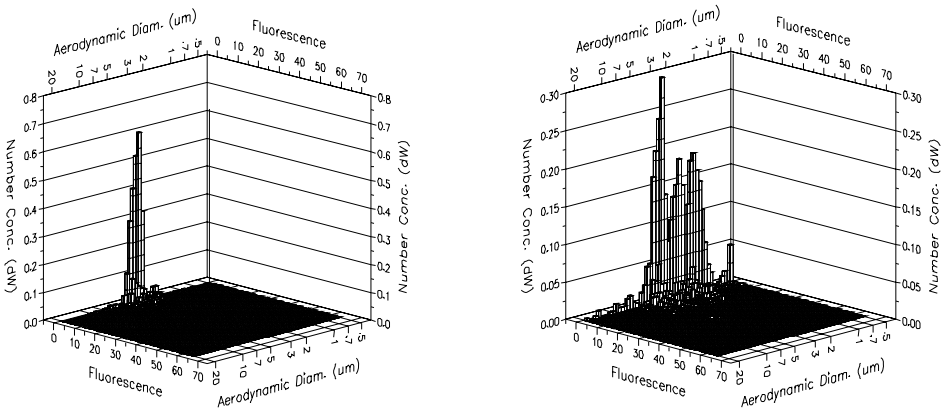
2005; Kanaani, Hargreaves et al. 2008). Table 1 presents the percentages of fungal fragmentation for different air velocities and generation methods. The fragmentation percentage is defined as the number of samples which contained fragmented spores as a percentage of the total number of samples at each air velocity level. While fungal fragmentation began at an airspeed of 1.8 m/s, for both the fan and FSSST methods, it started at 3.3 m/s for the direct method, for all of fungal species under investigation (Table 1). The percentage of fungal fragmentation, using the fan and FSSST methods, was much higher than using the direct method, for all air velocity rates. For instance at an air velocity of 5.3 m/s, the percentage of fungal fragmentation of *Penicillium*, *Aspergillus* and *Cladosporium* was 27.0, 10.5 and 14.5% for fan method, 24.3, 10.4 and 11.2% for the FSSST method and only 0.5, 0.3 and 6.5% for the direct method for, respectively. This may be explained by the perpendicular angle of the airflow to the culture, for both the fan and FSSST methods, which may have created more turbulence and thus, a greater percentage of fragmentation and fungal spore release (Górny, Reponen et al. 2001).



(a) *Penicillium species*



(b) *Aspergillus*



(c) *Cladosporium*

Figure 2. Typical UVAPS spectra for non-fragmented (left side) and fragmented fungal species (right side): (a) *Penicillium species* (ACM 4616); (b) *Aspergillus niger* (ATCC 9142); (c) *Cladosporium cladosporoides* (FRR 5106).

In general, the fragmentation percentage of the fungi increased with increasing air velocity (Table 1). However, there were two exceptions using the direct method, one for *Penicillium* (when the air velocity increased from 1.8 to 3.3 m/s) and the other for *Aspergillus* (when the air velocity increased from 3.3 to 5.5 m/s) and one exception using the fan method, for *Penicillium* (when the air velocity increased from 1.8 to 3.3 m/s). The reasons behind these exceptions are unclear.

The Mann-Whitney U test demonstrated that there were significant differences ($p < 0.01$) in the fragmentation percentage between 1.8, 3.3 and 5.3 m/s air velocities, for the three generation methods. The Kruskal-Wallis non-parameter test also found significant differences between the median scores (percentage of fragmentation) for each subsequent increase in air velocity (according to species and generation method). As such, each test was then followed by a Mann-Whitney U test and the differences remained significant ($p < 0.05$), except in two cases for *Penicillium*, where the differences were not significant (when the air velocity shifted from 1.8 to 3.3 m/s using the fan method ($p = 0.97$) and when the air velocity shifted from 3.3 m/s to 5.3 m/s using the direct method ($p = 0.14$).

This increase in fragmentation percentage and number of released propagules, as a function of air velocity, is consistent with the findings of previous studies (Górny, Mainelis et al. 2003; Górny 2004). Górny et al (2003) reported a 14 fold increase in the release of fragments in the actinomycete *Streptomyces albus* when increasing the air velocity on the agar surface from 0.3 m/s to 29.1 m/s. Górny et al. (2002) also found a noticeable increase in the number of released fragments for *Aspergillus versicolor*, *Penicillium melinii*, and *Cladosporium cladosporioides* from the ceiling

tile surfaces when air velocity over colonies was shifted from 0.3 m/s to 29.1 m/s. However, this increase was not observed when malt extract agar surfaces were used.

Table l. Percentage of fungal fragmentation as a function of air velocity and generation method

		Percentage of frequency fragmentation		
Method	Air velocity (m/s)	*Penicillium*	*Aspergillus*	*Cladosporium*
Fan	0.1	0.0	0.0	0.0
	0.4	0.0	0.0	0.0
	1.8	16.4 ± 5.3	5.5 ± 6.2	6.9 ± 4.2
	3.3	15.1 ± 4.2	6.5 ± 4.7	8.1 ± 5.3
	5.3	27.0 ± 7.6	10.5 ± 9.3	14.5 ± 7.9
FSSST	0.1	0.0	0.0	0.0
	0.4	0.0	0.0	0.0
	1.8	2.1 ± 2.0	0.9 ± 1.2	3.4 ± 2.1
	3.3	6.8 ± 4.1	4.1 ± 3.9	6.0 ± 3.8
	5.3	24.3 ± 9.3	10.4 ± 6.2	11.2 ± 6.1
Direct	0.1	0.0	0.0	0.0
	0.4	0.0	0.0	0.0
	1.8	0.0	0.0	0.0
	3.3	0.9 ± 0.9	0.8 ± 1.1	3.8 ± 2.1
	5.3	0.5 ± 0.45	0.3 ± 0.5	6.5 ± 3.0
	7.1	2.5 ± 2.3	0.7 ± 0.7	10.0 ± 4.8
	8.5	7.6 ± 6.9	7.3 ± 5.3	12.7 ± 7.1
	10.2	13.0 ± 10.2	11.1 ± 8.1	13.2 ± 6.9

Overall, the study found a significant correlation between fungal fragmentation percentage and air velocity for all species and generation methods, except for *Penicillium* (using the fan and FSSST methods) the correlation were not significant, where the p-value was slightly higher than 0.05: direct method: ($r^2 = 0.78$, $p < 0.05$; $r^2 = 0.71$, $p < 0.05$ and $r^2 = 0.94$, $p < 0.001$ for the direct method for *Penicillium*, *Aspergillus* and *Cladosporium,* respectively; $r^2 = 0.90$, $p < 0.05$ and $r^2 = 0.92$, $p < 0.05$ for the fan method; and $r^2 = 0.92$, $p < 0.05$ and $r^2 = 0.97$, $p < 0.005$ for the FSSST method, for *Aspergillus* and *Cladosporium,* respectively). The above results also demonstrated a more significant association between fungal fragmentation and air velocity for *Cladosporium*, compared to *Penicillium* and *Aspergillus*, for all generation methods. This may be explained by the ellipsoidal to limoniform shape of *Cladosporium* spores (Samson and Hoekstra 1994), which fragment more easily than the globose shape of *Penicillium* and *Aspergillus* spores (Raper, Fennell et al. 1965; Ramirez 1982). Table 1 also showed that the fragmentation percentage of *Cladosporium* was more abundant than that of *Penicillium* and *Aspergillus*, under the same conditions. The effect of the conidiophores shape for *Penicillium* (smooth-walled and singlet or branched), *Aspergillus* (smooth-walled and singlet) and *Cladosporium* (erect and straight unbranched or branched) (Ellis et al., 1992), together with the effect of their colony surfaces, on spore fragmentation also need to be investigated.

All of the strains, for each generation method, showed zero fragmentation at 0.4 m/s, indicating that for a typical indoor ventilation environment, where air velocity is ≤ 0.4 m/s (Thorshauge 1982; Matthews, Thompson et al. 1989; Handa and Pietrzyk 1996), there would be no fungal spore fragmentation for the species under investigation, except that which occurs in the ventilation systems (ducts, pipes etc.), where the air

speed can reach up to 29.1 m/s (Górny, Reponen et al. 2002). While there was a low concentration of spores released at 0.1 and 0.4 m/s for *Penicillium* (around 0.7 and 1.7 #/cm^3, respectively) and *Aspergillus* (around 0.09 and 0.15 #/cm^3, respectively), there were no spores released (0 #/cm^3) for *Cladosporium*. These results are consistent with a previous study by Pasanen et al. (1991), which reported that *Cladosporium* spores required a velocity of at least 1.0 m/s to be released. However, Pasanen et al. (1991) also demonstrated that the release of *A. fumigatus* and *Pencillium* sp. spores from conidiophores was initiated at an air velocity of 0.5 m/s, whereas this study found that they are released at a lower air velocity. This may due to the use of different *Pencillium* and *Aspergillus* species. In contrast, Górny et al. (2002) found fungal fragments for *Aspergillus versicolor*, *Penicillium melinii* and *Cladosporium cladosporioides* at 0.3 m/s. Again, this may due to the different species (except *Cladosporium cladosporioides*) and agar which were used, as well as the different sensitivities of the equipment used for measuring air velocity. It may also be due to the lower relative humidity observed in Górny et al. (2002) (32-40%) (compared to 65-69% in this study), since fungal propagules have been shown to be aerosolised more easily in dry air (Pasanen, Pasanen et al. 1991; Foarde, Dulaney et al. 1993).

3.2. The characterisation and mechanism of fungal spore fragmentation

In the previous study (Kanaani, Hargreaves et al. 2008), the concentration of released fungal particles was found to remain the same or decrease very slowly for successive samples of 20 seconds each. As such, any significant sudden change in concentration of particle number was deemed to be the result of fragmentation. A typical example of fragmentation and non-fragmentation for *Penicillium* is presented in Figure 3.

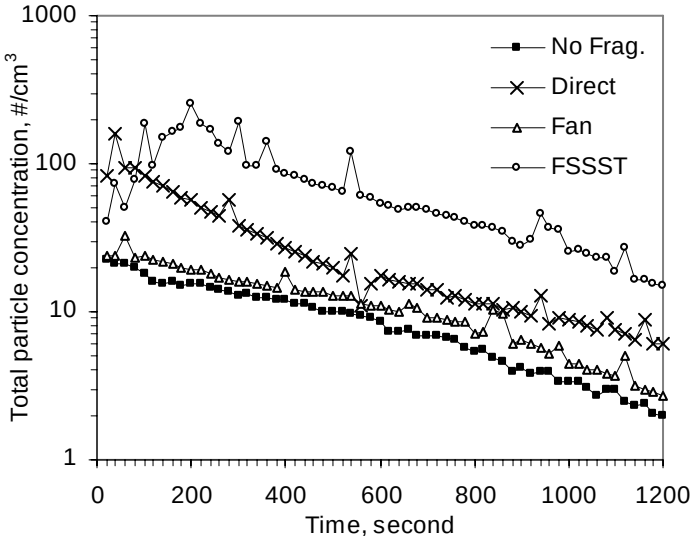


Figure 3. The frequency of fragmentation of 60 successive samples (20 seconds each) for different aerosolisation methods for *Penicillium* species. The 'No Frag.' data are presented for comparison purposes and are derived from using the fan method at 5.3 m/s.

As presented in Figure 3, 60 *Penicillium* samples were used to inve the frequency of fragmentation for the three generation methods, at the highest air velocity (direct method at 10 m/s, fan and FSSST methods at 5.3 m/s). The highest air velocity for each method was chosen as it gave the greatest percentage of fragmentation. The figure shows that there is no apparent pattern to the occurrence of fungal fragmentation, with frequencies ranging from 'interval' (many samples without fragmentation followed by one fragmented sample) to 'successive' (repeating fragmented samples ranging from 2-5 occurrences) and even 'no fragmentation for an extended period' (no fragmentation in more than 100 successive samples). Since temperature, humidity, air velocity, fungal generation method and species were all constant, other factors such colony orientation or the species strains and thickness of the conidiophores for each species may be responsible for the frequency of fragmentation. Further investigations are necessary to determine the reasons behind this phenomenon.

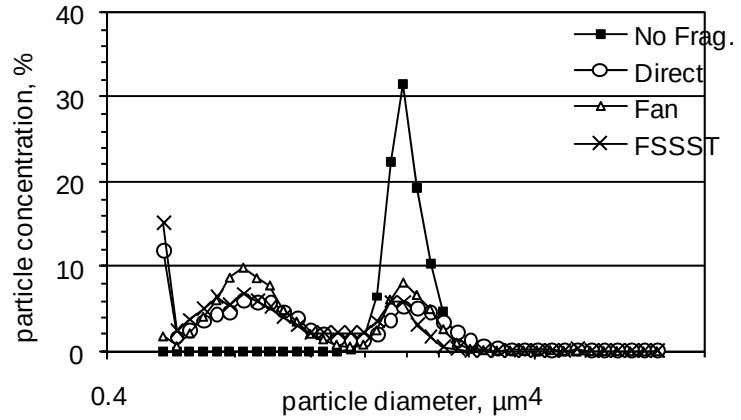
Figure 4 shows *Penicillium*, *Aspergillus* and *Cladosporium* gave multimodal particle size concentrations (3, 3 and 4 modes, respectively) when they fragmented, for each of the three generation methods used. These modes were 0.54, 0.97 and 2.28 μm for *Penicillium*, 0.54, 1.38 and 3.27 μm for *Aspergillus* and 0.54, 1.48, 2.28 and 3.27 μm for *Cladosporium*, using the direct, fan and FSSST methods, respectively. It was also found that the size of the modes of fragmented samples were associated with the mode size for the non-fragmented samples (i.e. the larger the non-fragmented origin particles, the larger the fragmented particles). For example, fragmented *Aspergillus* particles with a mode of 1.8 μm (1.4-2.6 μm) corresponded with non-fragmented origin particles with a mode of 3.3 μm (spore rang of 2.8-4.4 μm), while smaller fragmented particles with a mode of 1.3 μm (0.9-1.6 μm) corresponded with smaller non-fragmented original particles with a mode of 2.8 μm (2.5-3.7 μm). In both cases, the size difference between the fragmented and non-fragmented samples for each mode was 1.5 μm.

Figure 4 also shows that, in the case of both *Penicillium* and *Aspergillus*, the particle size distribution modes were similar for each of the generation methods used for that species, which suggests a specific fragmentation mechanism for each of these species. *Penicillium* and *Aspergillus* are very closely related imperfect fungi of the same subclass (Griffin 1994) and both of them have round spores (Larone 2002), the major difference being that *Aspergillus* spores are larger than *Penicillium* spores (which therefore lead to larger modes after fragmentation, as described above) with longer conidiophores and a rougher colony surface (Ellis, Davis et al. 1992). Since larger fragmentation modes were formed by *Aspergillus* than by *Penicillium* (Figure 4), this suggests that the fungal particles which fragmented were in fact spores (more proves are listed, below, in this section).

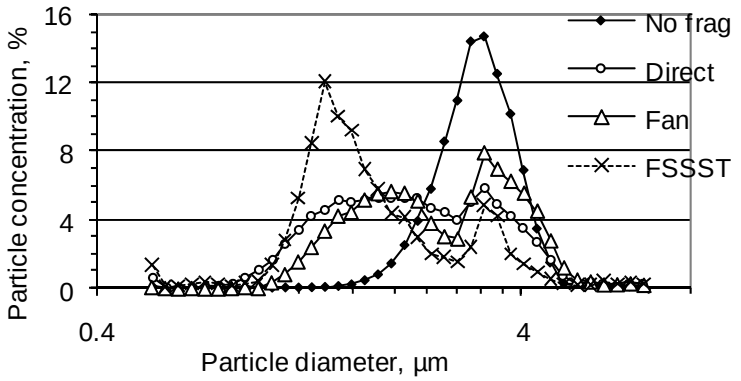
Using the direct method of generation, at an air velocity of 3.3 m/s, the distribution was bimodal for *Penicillium* and *Aspergillus* and trimodal for *Cladosporium*, with none of the species displaying a submicrometer mode (i.e. 2.6 μm and 1.4 μm for *Penicillium*, 3.52 μm and 1.71 μm for *Aspergillus* and 3.5 μm, 1.6 μm and 1.3 μm for *Cladosporium*) (Figure not shown). These results indicated that air velocity not only affects the percentage of sample fragmentation, but it also affects the fragmentation mechanism.

An example of a typical individual sample for each species is shown in Table 2. As shown in Table 2, the concentration of larger particles (spores) decreased after fragmentation, while the concentration of smaller particles increased, for all of the species investigated. This may due to the fact that the large spores have a larger surface area that is exposed to the air current and therefore, they fragment more easily than the small spores.

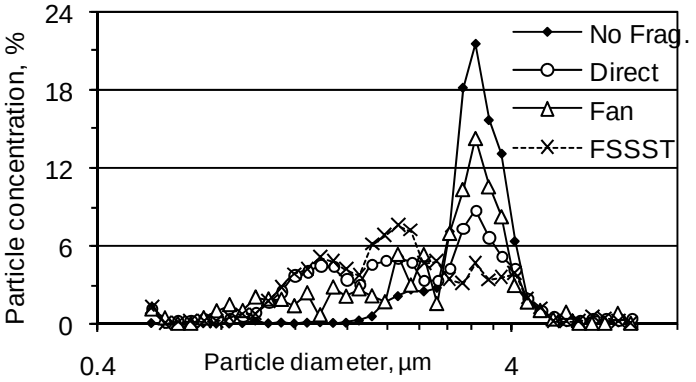
Table 2 also shows that after fragmentation, the large spores decreased in number and the smaller ones increased (the small spores were mostly 0-4 before fragmentation). Directly following fragmentation, the same mode and percentage of larger spores, as in the after fragmentation sample, were also detected in the following non-fragmented sample. On the other hand, the smaller particles which formed during fragmentation disappeared, which means that the particles that underwent fragmentation were spores. While, our results support the findings of Górny et al. (2002), who found that fragment samples of *Aspergillus* and *Penicillium* species share common antigens with their spores, which confirm the fungal origin of fragments, it also suggest spores as the source of fungal propagules which produce these fragments.



(a) *Penicillium*



(b) *Aspergillus*



(c) *Cladosporium*

Figure 4. Average particle concentration (for all 10 high fluorescentpercentage experiments) of fragmented and non-fragmented fungal species for each of the three generation methods: (a) *Penicillium*; (b) *Aspergillus*; (c) *Cladosporium.*

Table 2. Typical samples of fungal spores under investigation; before and after fragmentation.

	Penicillium		*Aspergillus*		*Cladosporium*	
A. D.[a]	N. F. S.[b]	F.S.[c]	N. F. S.	F.S.	N. F. S.	F.S.
0.54	0	4	1	12	0	12
0.58	4	273	0	0	0	1
0.63	0	71	0	1	0	0
0.67	0	99	1	1	0	3
0.72	0	202	0	2	0	7
0.78	0	368	0	0	0	7
0.84	0	507	1	2	0	4
0.90	0	849	1	2	0	0
0.97	0	946	1	0	0	8
1.04	0	982	0	12	0	15
1.11	2	898	1	27	0	22
1.20	0	837	0	52	3	36
1.29	0	528	1	113	1	51
1.38	2	421	1	196	2	46
1.49	4	273	1	292	1	32
1.60	2	200	0	315	1	16
1.72	7	126	0	383	3	11
1.84	42	93	1	333	3	18
1.98	281	152	1	298	10	12
2.13	1146	359	2	207	14	20
2.29	2710	845	3	168	11	14
2.46	2480	867	3	75	13	9
2.64	1642	650	30	80	21	12
2.84	1058	534	141	138	64	52
3.05	404	333	483	402	159	134
3.28	131	183	747	551	165	143
3.52	56	93	688	463	131	76
3.79	19	51	562	393	82	52
4.07	12	20	606	411	39	33
4.37	17	30	481	336	21	15
4.70	14	18	223	154	8	5
5.05	11	22	60	45	6	6
5.43	11	21	20	21	0	0
5.83	9	19	5	5	0	1
6.26	11	15	4	6	2	3
6.73	4	24	4	6	0	2
7.23	4	20	6	7	3	4
7.77	3	9	4	7	2	8
8.35	6	8	3	5	0	2
8.98	3	5	9	7	0	1
9.65	4	13	1	4	0	1
10.37	3	4	1	5	1	0

[a]Aerodynamic Diameter as measured by UVAPS.

[b]Not fragmented sample.

[c]Fragmented sample.

(The large spore numbers for each species (shaded area) were decreased after fragmentation.)

Fragmentation can lead to an increase in smaller particles by up to 5000 times (as found in this study). The number of fragments depends on many factors, such as initial concentration of the sample, air velocity, method of generation, fungal species and the number of particles of interest before fragmentation (in this case it was the smaller particles, which ranged between 0 - 4 particles per sample before fragmentation). For example, a direct generation sample of *Aspergillus*, with an initial concentration of $4.4x10^2$ #/cm^3 and an initial particle count of $1.44x10^5$ particles before fragmentation, was found to have only 1 particle of 1.84 μm in diameter before fragmentation, whilst the final concentration was found to be $5.6x10^2$ #/cm^3 and the final particle count was $1.84x10^5$after fragmentation, with $4.67x10^3$ particles of 1.84 μm in diameter. This increase was among the highest found in this study.

Overall, the concentration and number of particles during this study increased after fragmentation by a range of 1.01-1.92 times, depending on the conditions under which the fragmentation occurred. For example, the mean increase in concentration and particle number after fragmentation for all air velocities using the direct method, were 1.41 ± 0.27 and 1.39 ± 0.25 times for *Penicillium*, 1.34 ± 0.24 and 1.33 ± 0.25 times for *Aspergillus*, and 1.21 ± 0.26 and 1.22 ± 0.24 times for *Cladosporium*.

Each of the large spores in an individual sample was found to have different fragment spore percentages when compared to other spores in the same sample. The fragment spore percentage is defined as the number of fragmented spores of a certain size as a percentage of the non-fragmented spores of the same size in the original sample. To estimate the spore fragment percentage in each sample, two assumptions were made: firstly, that the spore fragment percentage of any spore size which increased in number after fragmentation is negligible; and secondly, that any large spores of certain size found in the fragmented sample represent the spores that did not fragment.

For example, for *Penicillium* (see Table 2), assumption one would meant that for 1.7 μm spores, an increase from 7 to 126 fungal particles after fragmentation would give a spore fragment percentage of 0.0 % (i.e. none of the original 7 spores underwent fragmentation), while the second assumption would mean that for 1.98 μm spores, the decrease from 281 to 152 spores after fragmentation would give a spore fragment percentage of 45.9% (i.e. the 152 spores that remain after fragmentation are assumed to be unfragmented spores). Even though assumption one may underestimate the spore fragment percentage and assumption two may overestimate it, given that some or all of the post-fragmentation spores may actually have originated from fragmented larger spores, Table 2 shows that in this study, the first assumption is valid because most of smaller sizes had a particle concentration of 0.0 before fragmentation. Similarly for the second assumption, this was relevant only for the larger spore sizes, which means that there would be very little contribution from fragmented larger spores. The mean spore fragment percentage ranges of *A. niger* at direct method at air velocities of 10.2 m/s, fan at 5.3 m/s and FSSST at 5.3 m/s were 23-65%, 27-72% and 40-86%, respectively with high fragmentation percentage were found, mostly, for mode spores and/or those close to the mode (i.e. one size above or below), for all of the species investigated.

The fragmentation percentage of spores is defined as the number of fragmented spores as a percentage of the total number of spores in the non-fragmented sample. The mean fragmentation percentage of spores (mostly, the same diameters as those shaded spore sizes in the examples shown in Table 2) at the highest air velocity applied in the study (10.2 m/s for the direct method and 5.3 m/s for the fan and FSSST methods) were 61.6, 50.0 and 45.2% for the direct method, 53.0, 40.2 and 31.9% for the fan method, and 70.9, 53.3 and 53.4% for the FSSST method, for *Penicillium Aspergillus* and

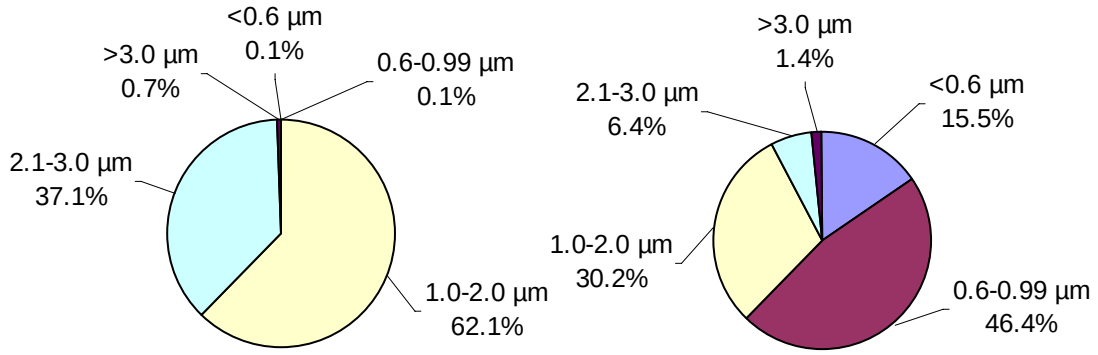
Cladosporium, respectively. From these figures it can be seen that the spore fragmentation percentages for the FSSST method were greater than for the other methods, for all of the species investigated. This may be due to the fact that the colonies generated using the FSSST method were under a small negative pressure (of a few Pascals), which may potentially lead to a greater fragmentation percentage for those spores.

When only using the UVAPS, it was difficult to determine if each spore of the investigated species was fragmented into three (or more) smaller parts, because if this was so, submicrometer particles, total particle number and sample concentration would dramatically increase, and this was not observed. Instead, it is hypothesised that each spore fragmented into two larger parts, because while the particle number and sample concentration increased in the range of 1.01-1.92, the total number of smaller particles did not reach double the number of total fragmented spores. For instance, the mean number of smaller particles which formed using direct method were 1.3 ± 0.31 times, 1.45 ± 0.26 times and 1.36 ± 0.42 times more than fragmented spores for *Penicillium Aspergillus* and *Cladosporium* at 10.2 m/s, respectively. This suggests that the fragmented spores did not divide into more than two large parts and that some of these spores fragmented so completely that they could not be detected by the UVAPS (they were only detected by the SMPS) (see Section 3.6.)

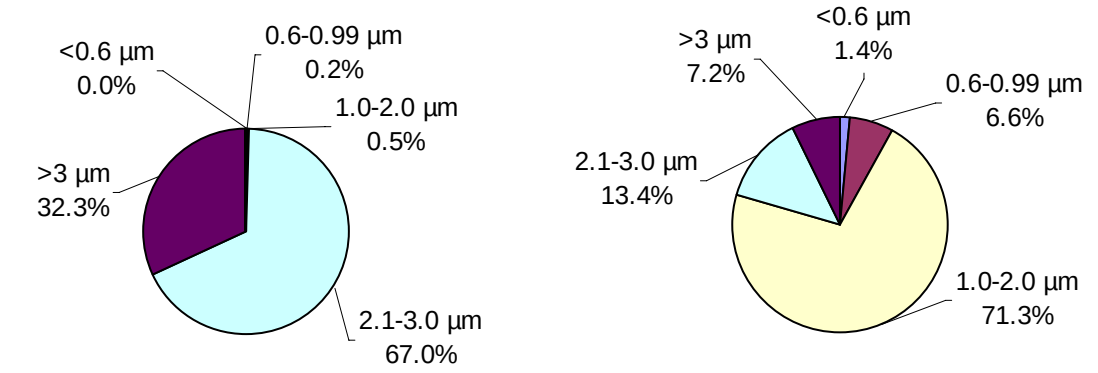
3.3. The expected impact of spore fragmentation on health

As shown in Figure 5, the largest portion of the fragmented sample for *Penicillium* (46.4%) was in the range 0.6-0.99 μm, while for *Aspergillus* and *Cladosporium* the largest portion of the sample (71.3% and 43.9%, respectively) was in the range 1-2 μm. Due to fragmentation, fungal particles (spores) larger than 3 μm decreased

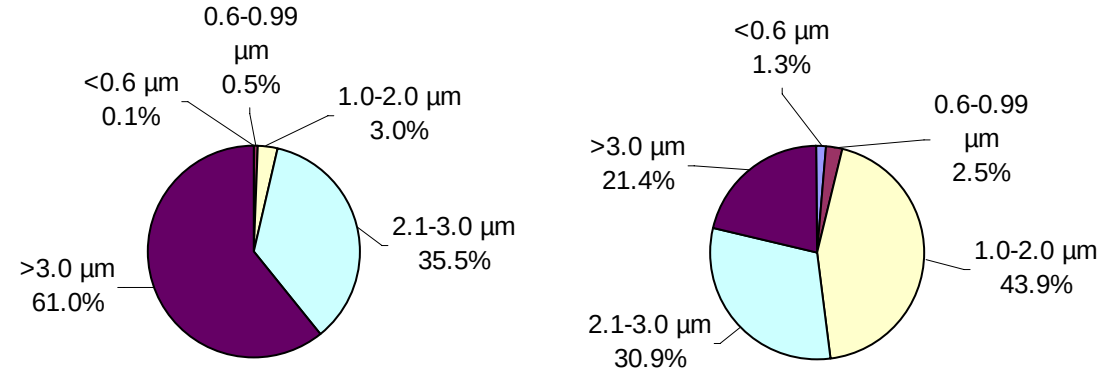
significantly after fragmentation for both *Aspergillus* and *Cladosporium*, while the submicrometer particles (which have a greater impact on allergic conditions) increased 400 times, 9.4 times and 6.3 times for *Penicillium*, *Aspergillus* and *Cladosporium*, respectively. Since Cho et al. (2005) showed that submicrometer fungal fragments are more likely than larger fragments to be deposited in the alveolar region of the lung, especially in young children, then it is expected the percentage deposition for *Penicillium* fragments in alveolar region of the lung would be greater than that of *Aspergillus* and *Cladosporium,* under comparable conditions. As fungal fragments have also been demonstrated to contain mycotoxins (Brasel, J. M. Martin et al. 2005) and fungal antigens (Górny, Reponen et al. 2002), this increase in submicrometer fungal particles may be a significant contributor to the adverse health effects associated with airborne fungal particles. As shown in Figure 5, spore fragmentation also led to an increase in the percentage of fine particles (< 2.5 μm) and these particles are more strongly associated with adverse health effects than coarse particles (Dockery, Pope et al. 1993; Levy, Hammitt et al. 2000). For the above reasons, fungal fragmentation needs to be taken into consideration when conducting future exposure measurements.



(a)



(b)



(c)

Figure 5. The particle concentration percentage of fungal particles with (right side) and with out (left side) fragmentation as detected by UVAPS: (a) *Penicillium*; (b) *Aspergillus*; (c) *Cladosporium*.

3.4. The correlation between spores and fragment release

Correlation analysis between the number of the spores and fragment release was conducted for all species for each generation method at the highest air velocity (10.2 m/s for the direct method and 5.3 m/s for fan and FSSST methods). The results demonstrated a statistically significant correlation (for the direct and FSSST methods) between *Penicillium* and *Aspergillus* fragments, and the spores detected by the UVAPS (r^2 = 0.65, 0.30 and 0.52 for *Penicillium* and r^2 = 0.77, 0.26 and 0.50 for *Aspergillus,* using the direct, fan and FSSST methods, respectively (in all, cases $p <$ 0.05). However no correlation was found between the spores and the fragments of *Cladosporium*, with the correlation coefficients found to be very low for all of the generation methods (r^2 = 0.13, p = 0.47; r^2 = 0.09, p = 0.65 and r^2 = 0.15, p = 0.70 for the direct, fan and FSSST methods, respectively). Previous studies have shown that the number of released fragments can not be predicted on the basis of the number of spores released (Górny, Reponen et al. 2002; Górny, Mainelis et al. 2003), however, the methods used in these studies were not the same for each set of experiments. Further, these studies classified the fragments for all species as being between 0.3-1.6 μm, while in this study, fragmented sizes were determined for each individual species and were found to range from 0.54-1.6 μm, 0.54-1.98 μm and 0.54-1.84 μm for *Penicillium*, *Aspergillus* and *Cladosporium*, respectively. In addition, the previous studies only looked at 30-min fungal propagule release (the time of each experiment), while the correlation between spores and fragments in this study were analysed for each sample (sample time 20 seconds).

3.5. Fungal particle fluorescent percentage

In all species and for all methods, the fluorescent percentage of the fragmented samples was lower than for the non-fragmented samples. For instance, using the fan method, the fluorescent percentage of the fragmented samples was lower than that of the non-fragmented samples by around 19.3%, 30.9% and 39.1% for *Penicillium* and 30.7%, 36.7% and 41.1% for *Aspergillus*, for high, medium and low fluorescent percentages, respectively. The fluorescent percentage of fragmented samples was also 21.8% lower than that of non-fragmented samples for *Cladosporium,* for high fluorescent percentages (Table 3). Using the direct method, the decrease was similar to that of the fan method, but the decrease was much less using the FSSST method (i.e. 9.1%, 13.7% and 12.3% for *Penicillium*, *Aspergillus* and *Cladosporium,* for high fluorescent percentages, respectively). Statistical analysis (Mann-Whitney U test) showed that there were significant differences ($p < 0.0001$) between the fluorescent percentage of fragmented and non-fragmented samples, for all of the species investigated, which is likely to be because many of the fragmented particles were smaller than the threshold detection limit of the UVAPS. On the other hand, the fluorescent percentage of fungal propagules decreased with their decreasing size, for all species, and the submicrometer fragments were found to have the lowest fluorescent percentage (Figure 6). These results agree with the previous findings of Kanaani et al. (2007). It was also found that when the original fluorescent percentage was smaller, the decrease in fluorescent percentage was actually greater (i.e. the percentage decrease for low fluorescent species was greater than that of medium fluorescent species, which was greater than that of high fluorescent species).

Table 3. Fluorescent percentage of fungal species with and with out fragmentation (fan method).

Fungal species	Fluorescent percentage of non fragment samples	Fluorescent percentage of fragment samples
Penicillium	80.2 ± 1.7	65.0 ± 4.2
	50.1 ± 1.3	34.6 ± 4.8
	24.3 ± 1.4	14.8 ± 3.5
Aspergillus	58.2 ± 1.1	40.3 ± 5.8
	41.4 ± 0.9	26.2 ± 4.3
	23.6 ± 1.2	13.9 ± 2.0
Cladosporium	29.3 ± 1.5	22.9 ± 3.9

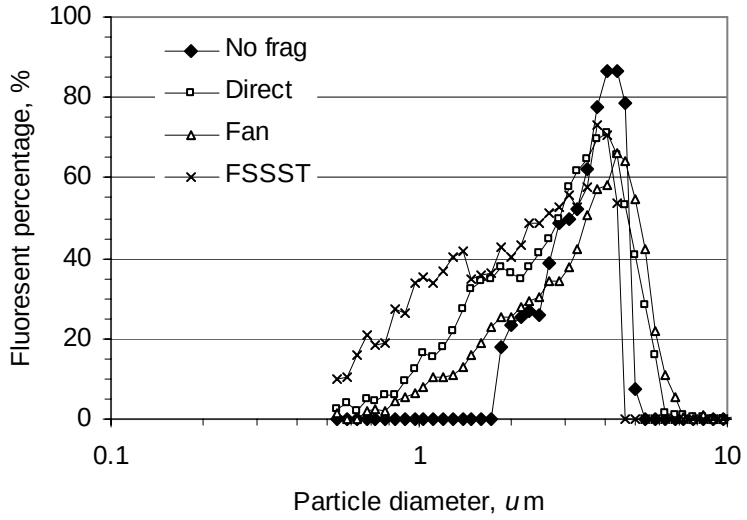


Figure 6. Fluorescent percentages of *Aspergillus* (high fluorescent) particles as a function of particle diameters.

3.6. Comparison of size distribution of fungal fragmentation particles as measures by UVAPS and SMPS

Fungal fragmented particles were detected simultaneously by the UVAPS and SMPS (Figure1). Most particle sizes (those less than 0.5 μm) that beyond the detection limits of UVAPS were detected by SMPS, so a full scale of fungal particles were collected

for both cases (fragmentation and not fragmentation). No signals were detected by the SMPS for the background, non-fragmented *Penicillium* or *Aspergillus* species in this study. Particle mobility diameter, d_m, can be related to its aerodynamic diameter, d_a, by introducing an effective density and it was corrected to aerodynamic diameter using the following equation (Shi, Harrison et al. 2001):

$$d_m = d_a \left(\frac{\rho_0 C_c(d_a)}{\rho_e C_c(d_m)} \right)^{0.5}$$

where $C_c(d)$ is the Cunningham slip correction, ρ_0 is unit density and ρ_e is the effective particle density. Fungal spore density of 1.1 g/cm^3 (Grinshpun, Mainelis et al. 2005) was used in this equation for ρ_e.

To compare similar particle sizes measured by the SMPS with that of the UVAPS, which located in the channels 0.542, 0.583, 0.626 and 0.673, the concentration of 533 and 552 nm particles detected in SMPS were summed together, since their mean was equal to 0.543 μm, which was comparable to channel 0.542 (μm) in the UVAPS. The same calculations were conducted for the following pairs of particle sizes as measured by SMPS: 573 and 594; 615 and 638; 661 and 685; and 710 and 737, in order to make comparisons with channel sizes 0.583, 0.626 and 0.673 in the UVAPS.

Fragments of all sizes were detected by the SMPS (down to 0.02 μm) for *Penicillium* and *Aspergillus,* however the number concentration percentage of ultrafine particle fragments (less than 0.1 μm) for *Penicillium* and *Aspergillus* were very low (3.5 ± 3.6 and 4.7 ± 5.1 %, respectively) when compared to the rest of the range (0.1-0.737 μm) measured by SMPS. Figure 7 shows a typical simultaneous sample for the SMPS and UVAPS. The figure shows that the UVAPS was more sensitive than SMPS for detecting particles of common diameters (0.54-0.723 μm). Generally speaking, it was

found that when a signal was detected by the SMPS, signal was also detected by the UVAPS, however the reverse was not always the case. This means that the sensitivity of the UVAPS to detect particles of certain diameter is greater than that of the SMPS, although, this sensitivity also depends on the diameter of the particles being detected. For example, the minimum concentration of 0.54 μm particles needed to be detected by the SMPS was greater than that needed for larger particles (i.e. 0.58, 0.63 and 0.72 μm). Further, while 3.5 #/cm^3 of 0.54 μm signal were detected by the UVAPS, no comparable signal was detected by the SMPS, however many exceptions were reported in both cases. For example, the SMPS was able to detect 0.1 #/cm^3 of 0.54 μm particles, while concentrations as high as 0.74, 1.37, 2.2 and 1.94 #/cm^3 for 0.58, 0.62, 0.67 and 0.723 μm particles detected by the UVAPS produced no signals in the SMPS. On the other hand, concentrations of 0.06, 0.1, 0.15 and 0.2 #/cm^3 for the same sized particles produced signals with the SMPS on numerous occasions. In order to determine the reasons behind these behaviours, and to quantify the total fragments detected in both SMPS and UVAPS, more detailed investigations need to be conducted in the future.

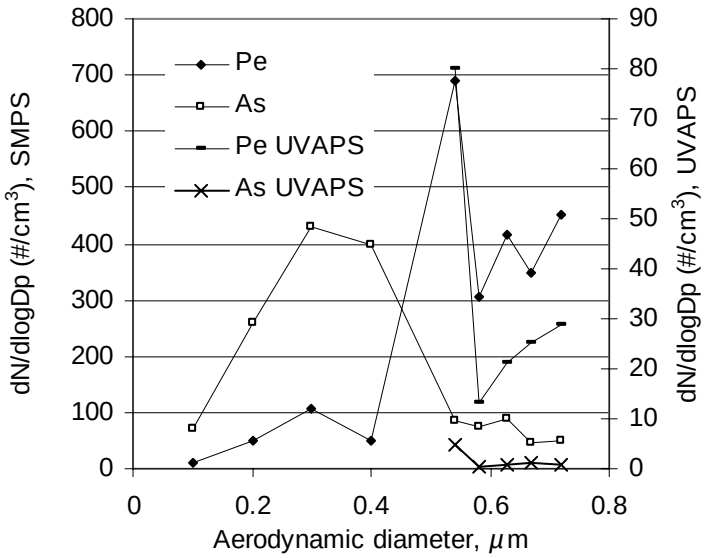


Figure 7. Typical samples of *Penicillium* and *Aspergillus* detected and measured by SMPS and UVAPS, simultaneously.

4. Conclusions

This study investigated the effects of using three different generation methods (direct, fan and FSSST), with different air velocity levels (0.1-10.2 m/s), on spore fragmentation mechanism. It also investigated the characterisation, and concentration of the released fungal fragments for that fungal spores (*Aspergillus*, *Penicillium* and *Cladosporium*), using a UVAPS. While most of the air velocity levels used in this study produced detectable fungal particles for all the three species and generation methods, no particle fragments were detected at 0.1 and 0.4m/s (i.e. the air velocities which represent normal indoor ventilation environments). The study showed a significant correlation between fungal fragmentation percentage and air velocity, (r^2 = 0.78, $p < 0.05$; r^2 = 0.71, $p < 0.05$ and r^2 = 0.94, $p < 0.001$ for the direct method for *Penicillium*, *Aspergillus* and *Cladosporium,* respectively; r^2 = 0.90, $p < 0.05$ and r^2 =

0.92, $p < 0.05$ for the fan method; and $r^2 = 0.92$, $p < 0.05$ and $r^2 = 0.97$, $p < 0.005$ for the FSSST method, for *Aspergillus* and *Cladosporium,* respectively). The specific parts of the fungal colony which were found to undergo fragmentation were the fungal spores. The results of this study also suggest that these fungal spores either fragmented into two larger particles, along with many smaller submicrometer particles or they fragmented into submicrometer particles only. Using the UVAPS, the submicrometer particles (which have a greater impact on health) were found to increase by up to 400 times, 9.4 times and 6.3 times for *Penicillium*, *Aspergillus* and *Cladosporium*, respectively, during fragmentation. It was also found that, for all species and methods, the fluorescent percentage of the fragmented samples was lower than that of the non-fragmented samples. On the other hand, the full scale of fungal particles produced for both fragmented and non-fragmented samples were detected, using a combination of UVAPS and SMPS, and the concentration of ultrafine particle fragments for *Penicillium* and *Aspergillus* were found to be very low when compared to the rest of the range (0.1-0.737 μm) measured by the SMPS.

Acknowledgements

This project was supported by Grant 1-9311-8174 from the Research Triangle Institute, Research Triangle Park, NC 27709-2194. Special thanks go to Dr David Ensor, Aerosol Science and Nanotechnology, whose vision made this study possible. The financial support Queensland University of Technology for one year is highly appreciated. The support provided by ILAQH staff and the assistance of senior research support specialist, Mr Ray Duplock, in relation to the statistical analysis performed in the study are also gratefully acknowledged.

References

Brasel, T. L., J. M. Martin, J. M., Carriker, C. G., Wilson, S. C.,Straus, D. C., 2005. Detection of Airborne Stachybotrys chartarum Macrocyclic Trichothecene Mycotoxins in the Indoor Environment. Applied and Environmental Microbiology 71(11): 7376-7388

Burge, H. A., Pierson, D. L., Groves, T. O., Strawn, K. F., Mishra, S. K., 2000. Dynamics of Airborne Fungal Populations in a Large Office Building. Current Microbiology (40): 10-16.

Burnett, J. H., 1976. Fundamentals of mycology. London, Edward Arnold.

Cho, S.-H., Seo, S.-C., Schmechel, D., Grinshpun, S. A., Reponen, T., 2005. Aerodynamic characteristics and respiratory deposition of fungal fragments. Atmospheric Environment 39(30): 5454-5465.

Dockery, D. W., Pope, C. A., Xu, X., Spengler, J. D., Ware, J. H., Fay, M. E., Ferris, B. G., Speizer, F. E., 1993. An association between air pollution and mortality in six U.S. Cities. The New England Journal of Medicine 329: 1753-1759.

Ellis, D., Davis, S., Alexious, H., Pfeiffer, T., Manatakis, Z., 1992. Descriptions of Medical QAP Fungi. Gillingham Printers Pty Ltd, Australia.

Foarde, K., Dulaney, P., Cole, E., VanOsdel, D., Ensor, D.,Chang, J., 1993. Assessment of fungal growth on ceiling tiles under environmentally characterized conditions. In: Kalliokoski, P., Jantunen, M., Seppänen, O. (Eds.) Particles, microbes, radon (Volume 4). Gummerus, Oy, Jyväskylä, Finland, Proceedings of Indoor Air 93 Conference. 4: 357-362.

Górny, R. L., 2004. Filamentous microorganisms and their fragments in indoor air - A review. Ann Agric Environ Med 11: 185-197.

Górny, R. L., Mainelis, G., Grinshpun, S. A., Willeke, K., Dutkiewicz, J.,Reponen, T., 2003. Release of Streptomyces albus propagules from contaminated surfaces. Environmental Research 91(1): 45-53.

Górny, R. L., Reponen, T., Grinshpun, S. A., Willeke, K., 2001. Source strength of fungal spore aerosolization from moldy building material. Atmospheric Environment 35: 4853-4862.

Górny, R. L., Reponen, T., Willeke, K., Schmechel, D., Robine, E., Boissier, M., Grinshpun, S. A., 2002. Fungal Fragments as Indoor Air Biocontaminants. Applied and Environmental Microbiology 68: 3522-3531.

Gregory, P., 1973. Microbiology of the atmosphere. John Wiley & Sons, Inc., New York.

Griffin, D. H., 1994. Fungal Physiology. John Wiley & Sons, Inc., New York.

Grinshpun, S. A., Górny, R. L., Reponen, T., Willeke, K., Trakumas, S., Hall, P., Dietrich, D. F., 2002. New method for assessment of potential spore aerosolization from contaminated surfaces. In Proceedings of the Sixth International Aerosol Conference, Taipei, Taiwan, 2: 767-768.

Grinshpun, S. A., Mainelis, G., Trunov, M., Górny, R. L., Sivasubramani, S. K., Adhikari, A., Reponen, T., 2005. Collection of airborne spores by circular single-stage impactors with small jet-to-plate distance. Journal of Aerosol Science 36(5-6): 575-591.

Handa, K., Pietrzyk, K., 1996. Full-scale measurements of indoor air flow. In Proceedings of the 17th AIVC conference optimum ventilation and air flow control in buildings. Air Infiltration and Ventilation Centre (AIVC), Great Britain, 1: 273-282.

Hargreaves, M., Parappukkaran, S., Morawska, L., Hitchins, J., He, C.,Gilbert, D., 2003. A pilot investigation into associations between indoor airborne fungal and non-biological particle concentrations in residential houses in Brisbane, Australia. The Science of The Total Environment 312(1-3): 89-101.

Jo, W.-K., Seo, Y.-J., 2005. Indoor and outdoor bioaerosol levels at recreation facilities, elementary schools, and homes. Chemosphere 61(11): 1570-1579.

Kanaani, H., Hargreaves, M., Ristovski, Z., Morawska, L., 2007. Performance assessment of UVAPS: Influence of fungal spore age and air exposure. Journal of Aerosol Science 38(1): 83-96.

Kanaani, H., Hargreaves, M., Smith, J., Ristovski, Z., Agranovski, V.,Morawska, L., 2008. Performance of UVAPS with respect to detection of airborne fungi. Journal of Aerosol Science 39(2): 175-189.

Kildesø, J., Würtz, H., Nielsen, K. F., Kruse, p., Willeke, K., Thrane, U., Gravesen, S., Nielsen, P. A., Schneider, T., 2003. Determination of fungal spore release from wet building materials. Indoor Air 13(2): 148-155.

Kuo, Y.-M., Li, C.-S., 1994. Seasonal fungus prevalence inside and outside of domestic environments in the subtropical climate. Atmospheric Environment 28(19): 3125-3130.

Larone, D. H., 2002. Medically important fungi a guide to identification. ASM Press, Washington, U.S.A.

Levy, J. I., Hammitt, J. K., Spengler, J. D., 2000. Estimating the mortality impacts of particulate matter: what can be learned from between-study variability? Environ Health Perspect 108(2): 109-117.

Madelin, T. M., Madelin, M. F., 1995. Biological analysis of fungi and associated molds. In Cox, C. S.,Wathes, C. M. (Eds.) Bioaerosols Handbook. CRC Press, Inc., Boca Raton.

Matthews, T. G., Thompson, C. V., Wilson, D. L., Hawthorne, A. R.,Mage, D. T., 1989. Air velocities inside domestic environments: An important parameter in the study of indoor air quality and climate. Environment International 15(1-6): 545-550.

Pasanen, A.-L., Pasanen, P., Jantunen, M. J., Kalliokoski, P., 1991. Significance of air humidity and air velocity for fungal spore release into the air. Atmospheric Environment. Part A. General Topics 25(2): 459-462.

Pei-Chih, W., Huey-Jen, S., Chia-Yin, L., 2000. Characteristics of indoor and outdoor airborne fungi at suburban and urban homes in two seasons. The Science of The Total Environment 253(1-3): 111-118.

Portnoy, J. M., Barnes, C. S., Kennedy, K., 2004. Sampling for indoor fungi. Journal of Allergy and Clinical Immunology 113(2): 189-198.

Ramirez, C., 1982. Manual and atlas of the penicillia. Elsevier Biomedical Press, Amsterdam.

Raper, K. B., Fennell, D. I., Austwick, P. K. C., 1965. The genus *Aspergillus*. Robert E. Krieger Publishing Co., Inc., U.S.A.

Samson, R. A., Hoekstra, E. S., 1994. Descriptions and illustrations of common fungi in indoor environments. In Samson, R. A., Flannigan, B., Flannigan, M. E. (Eds.) Health implication of fungi in indoor environments, Version 2. Elsevier Science B, Amsterdam.

Shi, J. P., Harrison, R. M., Evans, D., 2001. Comparison of ambient particle surface area measurement by epiphaniometer and SMPS/APS. Atmospheric Environment 35(35): 6193-6200.

Sivasubramani, S. K., Niemeier, R. T., Reponen, T., Grinshpun, S. A., 2004. Assessment of the aerosolization potential for fungal spores in moldy homes. Indoor Air 14: 405-412.

Sivasubramani, S. K., Niemeier, R. T., Reponen, T., Grinshpun, S. A., 2004. Fungal spore source strength tester: laboratory evaluation of a new concept. Science of The Total Environment 329(1-3): 75-86.

Smith, A. L., 1976. Microbiology and Pathology. The C. V. Mosby Company, Saint Louis.

Solomon, W. R., 1976. A volumetric study of winter fungus prevalence in the air of midwestern homes. Journal of Allergy and Clinical Immunology 57(1): 46-55.

Thorshauge, J., 1982. Air-velocity fluctuations in the occupied zone of ventilated spaces ASHRAE Transactions 88(Part 2): 753-764.

Vijay, H. M., Thaker, A. J., Banerjee, B., Kurup, V. P. (Eds.), 1999. Allergens and Allergen Immunotherapy. Marcel Dekker, Inc., New York.